BIOTECHNOLOGY OF EDIBLE MUSHROOMS CULTIVATION ON VINE AND WINERY WASTES

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Abstract. *Every year, in Romania huge amounts of wine and vine wastes cause serious environmental damages in vineyards as well as nearby winery factories, for instance, by their burning on the soil surface or their incorporation into soil matrix. The optimal and efficient way to solve these problems is to recycle these biomass wastes as main ingredients in nutritive composts preparation that could be used for edible mushrooms cultivation. In this respect, the main aim of this work was to establish the best biotechnology of winery and vine wastes recycling by using them as appropriate growth substrata for edible and medicinal mushrooms. According to this purpose, two mushroom species of Basidiomycetes, namely Lentinula edodes as well as Pleurotus ostreatus were used as pure mushroom cultures in experiments. The experiments of inoculum preparation were set up under the following conditions: constant temperature, 23°C; agitation speed, 90-120 rev min $^{-1}$; pH level, 5.0–6.0. All mycelia mushroom cultures were incubated for 120–168 h. In the next stage of experiments, the culture composts for mushroom growing were prepared from the lignocellulose wastes as vine cuttings and marc of grapes in order to be used as substrata in mycelia development and fruit body formation. The tested culture variants were monitored continuously to keep constant the temperature during the incubation as well as air humidity, air pressure and a balanced ration of molecular oxygen and carbon dioxide. In every mushroom culture cycle all the physical and chemical parameters that could influence the mycelia growing as well as fruit body formation of L. edodes and P. ostreatus were compared to the same fungal cultures that were grown on poplar logs used as control samples.*

Keywords*: biomass, composts, edible mushrooms*

Introduction

The agricultural works as well as the industrial activities related to wine crops and wine processing have generally been characterized by huge formation of wide range of waste products. Many of these lignocellulose wastes cause serious environmental pollution effects, if they are allowed to accumulate in the vineyards or much worse to be burned on the soil [1, 2]. The solid substrate fermentation of plant wastes from agro-food industry is one of the most challenging and technically demanding biotechnologies known to humankind [3-5]. The major group of fungi to degrade cellulose and lignocellulose are the edible mushrooms of *Basidiomycetes* Class [6-9]. The main aim of this work was to find out the best biotechnology of recycling winery and vineyard wastes by using them as a growing source for edible mushrooms and, last but not least, to protect the vineyard ecosystems [9-12].

Taking into consideration that most of the edible mushrooms species requires a specific micro-environment including complex nutrients, the influence of all physical and chemical factors upon fungal

biomass production and mushroom fruit bodies formation has been studied by testing new biotechnological procedures [7-12].

Materials and methods

According to the main purposes of this work, two fungal species of Basidiomycetes group, namely *Lentinula edodes* (Berkeley) Pegler (folk name: Shiitake) as well as *Pleurotus ostreatus* (Jacquin ex Fries) Kummer (folk name: Oyster Mushroom) were used as pure mushroom cultures isolated by authors from the natural environment and now being preserved in the local collection of the University of Pitesti.

The stock cultures were maintained on malt-extract agar (MEA) samples (20% malt extract, 2% yeast extract, and 20% agar-agar). Samples were incubated at 25°C for 120-168 h and stored at 4°C.

The pure mushroom cultures were expanded by growing in 250-ml flasks containing 100 ml of liquid malt-extract medium at 23°C on rotary shaker incubators at 110 rev. min $^{-1}$ for 72-120 h.

To prepare the inoculum for the spawn cultures of *L. edodes* and *P. ostreatus* the pure mushroom cultures were inoculated into 100 ml of liquid malt-yeast extract culture medium with 3-5% (v/v) and then maintained at 23-25°C in 250 ml rotary shake flasks.

The experiments of inoculum preparation were set up under the following conditions: constant temperature, 25°C; agitation speed, 90-120 rev. min $^{-1}$; initial pH, 5.5–6.5. All the seed mushroom cultures were incubated for 120–168 h.

After that, the seed cultures of these mushroom species were inoculated into liquid culture media (20% malt extract, 10% wheat bran, 3% yeast extract, 1% peptone) at pH 6.5 previously distributed into rotary shake flasks of 1.000 ml. During the incubation time period, all the spawn cultures were maintained in special culture rooms, designed for optimal incubation at 25^0C.

Three variants of culture compost made from marc grapes and vineyard cuttings in the following ratios: 1:1, 1:2, 1:4 (w/w) ere prepared.

The vine and winery wastes were mechanically pre-treated by using an electric grinding device to breakdown the lignin and cellulose structures in order to make them more susceptible to the enzyme actions [10-12].

All the culture compost variants made from ground vineyard and winery wastes were transferred into 1.000 ml glass jars and disinfected by steam sterilization at 120^0 C for 60 min.

After the jars filled with composts have been chilled, they were inoculated with liquid spawn already prepared. Each culture compost variant for mushroom growing was inoculated using liquid spawn having the age of 72–220 h and the volume size ranging between 3–9% (v/w).

During the period of time of 18–20 d after this inoculation, all the mushroom cultures had developed a significant mycelia biomass on the culture substrata made from vineyard cuttings and marc of grapes [10-12].

Results and discussion

According to the registered results of the performed experiments the optimal laboratory-scale biotechnology for edible mushroom cultivation on composts made from marc of grapes and vineyard cuttings was established. As it is shown in fig. 1, two technological flows were carried out simultaneously until the first common stages of the inoculation of composts with liquid mushroom spawn followed by the mushroom fruit body formation.

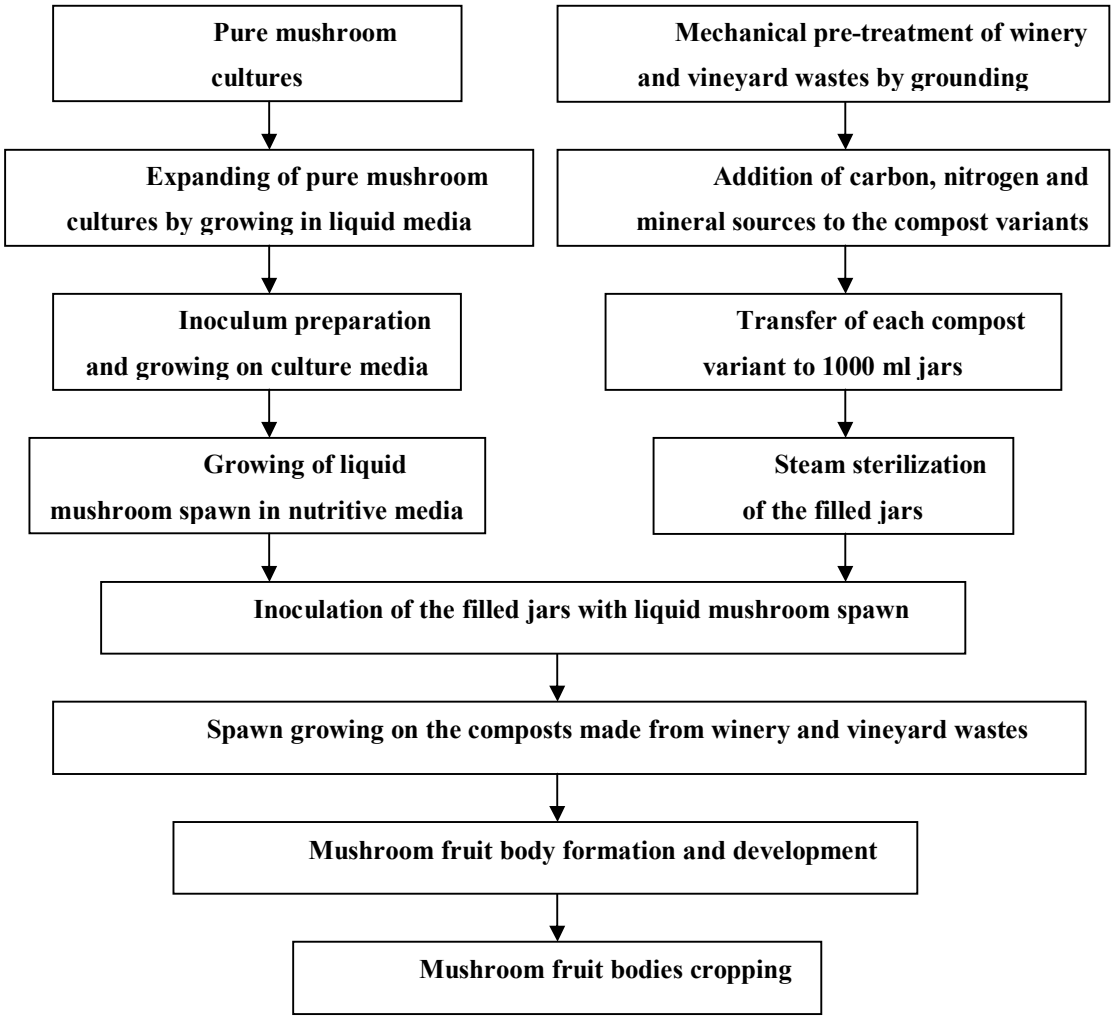

Fig. 1. Scheme of laboratory-scale biotechnology for edible mushroom production by recycling winery and vineyard wastes

Series of three cultivation cycles were made and the effects induced by some additional ingredients as carbon sources (xylose, sucrose, maltose, glucose) upon the mycelia growing during each cultivation period were investigated.

As it could be noticed in figure 2, each carbon source was added to the basal composts at a concentration level of 5% (w/w) and the incubation time period lasted for 168-288 h [12-14].

Maltose, as one of all mostly tested carbon sources, had shown the highest influence upon the mycelia growing and fresh fungal biomass production about of 28–35g%.

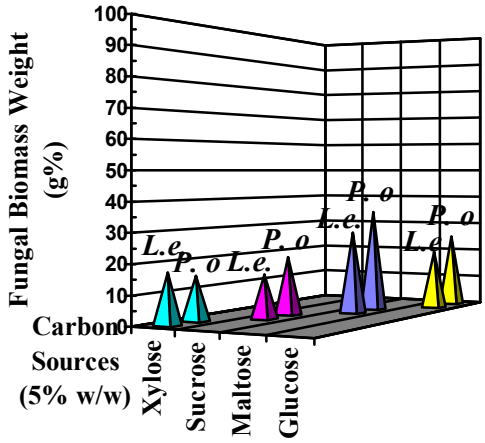

Fig. 2. Comparative effects of carbon sources upon mycelia growing of *P. ostreatus* (*P.o.*) and *L. edodes* (*L.e.*)

The effects of nitrogen sources were noticed and registered as they are shown in figure 3.

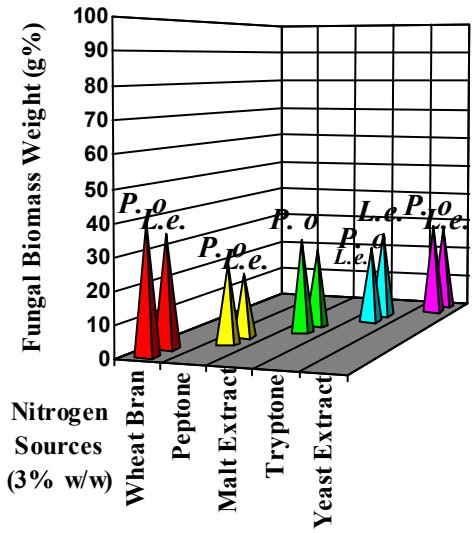

Fig. 3. Comparative effects of nitrogen sources upon mycelia growing of *P. ostreatus* (*P.o.*) and *L. eodes* (*L.e.*)

From all tested nitrogen sources (wheat bran, peptone, malt extract, tryptone and yeast extract), only the wheat bran was the most efficient upon the mycelia growing and fungal biomass producing at 35-40 g% fresh fungal biomass weight, being closely followed by the malt extract at 25–30 g%.

Peptone, tryptone and yeast extract are also well known as nitrogen sources for fungal biomass synthesis but their efficiency in these experiments was relatively lower than the mycelia growing and fungal biomass production induced by the wheat bran added as natural organic nitrogen sources [15-17].

Among the various mineral sources examined, such as: $CaCO_3$, $CaSO_4$, K_2HPO_4, $MgSO_4 \cdot 5H_2O$, KH_2PO_4, the first one, $CaCO_3$, had the best mycelia growing yielded. In this case, the fungal biomass production was registered at 28-32 g% and for this reason the most appropriate mineral source was $CaCO_3$ (Fig.4).

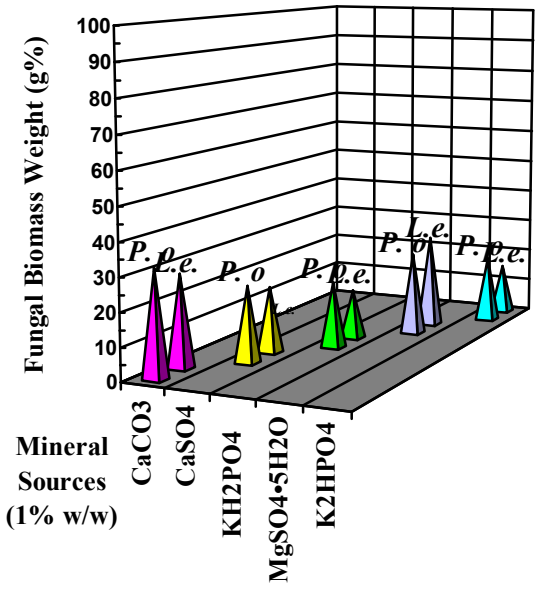

Fig. 4. Comparative effects of mineral sources upon mycelia growing of *P. ostreatus* (*P. o.*) and *L. edodes* (*L.e.*)

All the experiments were carried out for 288 h at 25°C with the initial pH 6.5 and data are the means of triple determinations carried out on the variants of composts made from vineyard cuttings and marc of grapes in the ratio 1:4.

Similar observations were made by Stamets (1993), during the experiments concerning other techniques of mushroom cultivation as well as other researchers [15-17]. Also, other tested mineral sources, such as $MgSO_4 \cdot 5H_2O$ have shown an optimal influence upon the fungal biomass growing [17-18].

The mineral sources K_2HPO_4 and KH_2PO_4 as essential phosphates could improve the pH level through their buffering action, but they were less appropriate for mycelia growing in submerged as well as in surface cultures of mushrooms.

The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30–60 d, depending on each fungal species used in the experiments.

During the whole period of fruit body formation, the culture parameters were set up and maintained at the following levels, depending on each mushroom species: air temperature, 15–17^{0}C; the air flow volume, 5–6m^3/h; air flow speed, 0.2–0.3 m/s; the relative moisture content, 80–85%, light intensity, 500–1.000 luces for 8–10 h/d. The final fruit body production of these mushroom species used in the experiments was registered between 1.5 – 2.8 kg relative to 10 kg of composts made from vineyard and winery wastes.

Conclusions

1. The registered data revealed that by applying this biotechnology, the winery and vineyard wastes could be recycled as useful raw materials for culture compost preparation to get edible mushrooms.
2. Maltose, as one of all mostly tested carbon sources, had shown the highest influence upon the mycelia growing and fresh fungal biomass production about of 28–35 g%;
3. Among the five nitrogen sources examined, wheat bran was the most efficient one upon the mycelia growing and fungal biomass production of *L. edodes* and *P. ostreatus*, at 35-40 g% fresh fungal biomass weight, closely followed by malt extract at 25–30 g%.
4. $CaCO_3$ was registered as the best mineral source, yielding the best mycelia growing as well as fungal biomass production at 28-32 g%.
5. The final fruit body productions of these two mushroom species were registered between 1.5–2.8 kg relative to 10 kg of composts made from vineyard and winery wastes.

Acknowledgements

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References

1. P. BEGUIN AND J.P. AUBERT, *FEMS Microbiol. Rev.*, **13**, 25 – 58 (1994).
2. M.J. CARLILE and S.C. WATKINSON, "Fungi and Biotechnology", in *The Fungi*, edited by M.J. Carlile and S.C. Watkinson, Academic Press, London, 1996, pp. 214-221
3. D.S. CHAHAL, "Biological disposal of lignocellulosic wastes and alleviation of their toxic effluents" in *Biological Degradation and Bioremediation of Toxic Chemicals*, edited by G.R. Chaudry, Chapman & Hall, London, 1994, pp. 347-356.
4. D.S. CHAHAL and J.M. HACHEY, *Am. Chem. Soc. Symp*., **433**, 304 – 310 (1990).
5. S.T. CHANG and W.A. HAYES, *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, 1978, pp. 114-143.
6. R.T. LAMAR, J.A. GLASER, T.K. KIRK, "White rot fungi in the treatment of hazardous chemicals and wastes", in *Frontiers in industrial mycology*, edited by G.F. Leatham, Chapman & Hall, New York, 1992, pp. 127-143.
7. J.G. LEAHY and R.R. COLWELl, *Microbial Rev.*, **54**, 305–315 (1990).
8. A. MOSEr, *Acta Biotechnology* **12**, 2-6 (1994).
9. M. PETRE, Biotechnology for microbial degradation and conversion of plant constituents, Ed. Didactica si Pedagogica, Bucuresti, 2002, pp. 140-214
10. M. PETRE, C. BEJAN, E. VISOIU, I. TITA, A. OLTEANU, *Intl. J. of Med. Mush.*, **9(3)**, 241-243 (2007).
11. M. PETRE, V. PETRE, *Journal of Environmental Protection and Ecology*, **9(1)**, 88-97 (2008).
12. M. PETRE, A. TEODORESCU, G. DICU, *Intl. J. of Med. Mush.* **7(3)**, 444-446 (2005).
13. M. ROPARS, R. MARCHAL, J. POURQUIE, J.P., VANDERCASTEELE, *Biores. Technol.*, **42**, 197-203 (1992).
14. J.E. SMITH, Biotechnology, Third Edition, Cambridge University Press, 1998, pp. 95-110.
15. P. STAMETS, Growing Gourmet and Medicinal Mushrooms, Ten Speed Press, Berkeley, Toronto, 1993, pp. 390-400.
16. W. VERSTRAETE and E. TOP, Holistic Environmental Biotechnology, Cambridge University Press, 1992, pp. 120-128.
17. M. WAINWRIGHT, An Introduction to Fungal Biotechnology, Wiley-Chichester, 1992, pp. 97-115.
18. G. ZARNEA, Tratat de microbiologie generala, vol 5, Ed. Academiei, Bucuresti, 1994, pp. 93-120.